

TESTING COMPETENCE OF CELLS

Aim

Testing competence of cells by transforming them with test plasmid with varying concentrations.

Procedure

1. Spin down the DNA tubes from the Competent Cell Test Kit. 20-30 seconds at 8000-10,000 rpm will be sufficient.
2. Thaw competent cells on ice. Label one 2.0 ml microcentrifuge tube for each concentration and then pre-chill by placing the tubes on ice => 5 tubes
3. Pipet 25 μ l of competent cells into each tube. Pipet 0.5 μ l of DNA into each microcentrifuge tube. Flick the tube gently with your finger to mix and add 5 μ L of KCM. Incubate on ice for 30 minutes. Pre-heat heating block now to 42°C.
4. Heat-shock the cells by placing into the heating block for 1 minute.
5. Immediately transfer the tubes back to ice, and incubate on ice for 5 minutes.
6. Add 100 μ L of SOC medium per tube, and incubate at 37°C for 1-2 hours on the shaker.
7. Prepare the agar plates during this time.
8. Pipet 20 μ L from each tube onto the appropriate plate, and spread the mixture evenly across the plate. Do triplicates (3 each) of each tube if possible, so you can calculate an average colony yield. Incubate at 37°C overnight or approximately 18 hours. Position the plates so the agar side is facing up, and the lid is facing down.
9. Count the number of colonies on a light field or a dark background, such as a lab bench. Use the following equation to calculate your competent cell efficiency. If you've done triplicates of each sample, use the average cell colony count in the calculation.
 - (colonies on plate) / ng of DNA plated x 1000 ng/ μ g
 - Note: The measurement "ng of DNA plated" refers to how much DNA was plated onto each agar plate, not the total amount of DNA used per transformation. You can calculate this number using the following equation:
 - 0.5 μ L x concentration of DNA x (volume plated / total reaction volume)

Note!

- Competent cells are very sensitive to both mechanical disruption and temperature changes. To mix cells, do not vortex but instead pipet up and down carefully and slowly. Always thaw cells on ice.
- When performing the heat shock, the timing is very important and the tubes should be kept very still.
- When growing transformed cells do not leave in the incubator for >16h due to the risk of satellite colonies developing. Plates can be left at RT or 4°C instead if colony picking can not be performed immediately.

Sources

http://parts.igem.org/Help:Competent_Cell_Test_Kit (retrieved 04.10.2016)